THE COMPOSITION OF

BERBERINE PHOSPHATE

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HAVING had occasion to prepare several salts of berberine, including the phosphate, and as some uncertainty seems to have prevailed regarding the composition of the latter, Dr. Power suggested to me that I undertake an investigation of the subject.

The history of berberine phosphate has been given by Lloyd,[1] who states that a substance was introduced into commerce under this name by Dr. T. L. A. Greve [2] about the year 1877. The substance prepared by Dr. Greve by the treatment of mono-berberine sulphate with precipitated calcium phosphate was proved, however, by Parsons and Wrampelmeier in 1878 to contain no phosphoric acid, and to consist of di-berberine sulphate.

Lloyd [3] described the preparation of the salt from berberine and phosphoric acid, and stated its solubility, as determined by Mr. Lord to be 1 part in 280 parts of water.

Parsons and Wrampelmeier[4] prepared the salt by heating together mono-berberine sulphate and acid calcium phosphate, $Ca H_4 (P O_4)_2$, evaporating nearly to dryness, extracting with hot diluted alcohol, again evaporating nearly to dryness, and treating with cold stronger alcohol. In the abstract of their paper (*loc. cit.*) it does not appear that the product thus obtained was examined for calcium, an impurity which is likely to occur, as will be shown later. From their analysis, apparently of the recrystallised salt, they were led to adopt the formula $C_{20} H_{17} N O_4 \cdot 7 H_3 P O_4 + 4 H_2 O.$[5]

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[1] *Drugs and Medicines of North America*, vol. i. p. 122.
[2] *Eclectic Medical Journal*, Cincinnati, 1877, p. 311.
[3] *Proc. Amer. Pharm. Assoc.*, 1878, p. 802.
[4] *Ibidem*, 1879, p. 514.
[5] In explanation of this formula, Parsons and Wrampelmeier remarked as follows:—" This formula seems, at first sight, an improbable one, but

Wilmarth[1] prepared a berberine phosphate by exhausting the powdered root of *Hydrastis canadensis* with water, evaporating, extracting with alcohol, again evaporating, and treating the aqueous solution of this residue with a large excess of dilute phosphoric acid. The crystals which separated from the concentrated liquid on standing were washed free from acid with 94 per cent. alcohol, and recrystallised from hot alcohol of the same strength. Parsons analysed this salt, and assigned to it the formula $C_{20}H_{17}NO_4 \cdot 2H_3PO_4$.

Schmidt (*Pharm. Chemie*, 3rd edit., Bd. ii., p. 1323) gives the formula $(C_{20}H_{17}NO_4)_3(H_3PO_4)_2 + 5H_2O$ for a berberine phosphate, which is stated to be prepared by adding to powdered berberine in hot water sufficient phosphoric acid to render the liquid slightly acid, concentrating, and precipitating the salt with alcohol. I have not been able to refer to any published analytical data relating to this formula.

In Lloyd's *Drugs and Medicines of North America*, 1885, p. 124, it is noted that the previously mentioned analysis by Parsons and Wrampelmeier being the only one known to them at that time of a compound of berberine and phosphoric acid, it seemed desirable to add further information to the subject. Accordingly, Prof. Virgil Coblentz was requested to undertake some analyses of the compound. Coblentz proceeded by dissolving a given weight of pure berberine in absolute alcohol, adding a known quantity of phosphoric acid, sufficient to ensure an excess, treating the alcoholic solution with absolute ether, filtering off the precipitate, and estimating the phosphoric acid in the filtrate. By this indirect method of estimating the combined phosphoric acid, which was conducted both gravimetrically and volumetrically, Coblentz arrived at the conclusion that the formula for the salt must be $C_{20}H_{17}NO_4 \cdot 7H_3PO_4$.

For my own investigation of the composition of berberine phosphate the salt was prepared by the interaction of berberine acetone

any person who will take the pains to look up the formulæ for the phosphates of the other alkaloids will be surprised at their lack of uniformity, and at the fact that alkaloids exhibit no particular quantivalence." Lloyd's *Drugs and Medicines of North America*, vol. i. p. 124. Dr. Power in *Contributions from the Department of Pharmacy of the University of Wisconsin*, 1885, No. 1, p. 52, remarked concerning the above formula: "Some experiments made by me with a phosphate of berberine prepared by Professor Lloyd, gave results which would lead to a very different formula for this salt, and which will be reported on at a later date."

[1] *Proc. Amer. Pharm. Assoc.*, 1879, p. 515.

(a compound which is easily obtained pure) with an excess of phosphoric acid, and recrystallising the product.

The salt thus prepared was of a bright yellow colour. It did not increase in weight even in a damp atmosphere.[1] The crystallised salt is soluble in 14·3 parts of water at 16° C., the dehydrated salt in 15 parts of water at 15° to 16° C.

The analysis of this salt was conducted as follows :—

(a) The water of crystallisation was determined by heating to 110° C. until of constant weight.

(b) For the determination of the phosphoric acid a weighed quantity of the salt was treated in a conical flask with about 5 c.c. of pure sulphuric acid, powdered potassium nitrate added, and the flask gently heated. After cooling, more potassium nitrate was added, and the liquid again heated until it was colourless, or only pale yellow, and the red fumes had disappeared. It was then diluted with water, boiled, and made alkaline with ammonia. The phosphoric acid was determined in this liquid by precipitating with magnesia mixture in the usual way.

(c) The base was determined by dissolving a known weight of the salt in hot water, adding a slight excess of hydrochloric acid, and then an excess of solution of platinic chloride, equal to about twice the amount required to form the platinum double salt. The liquid containing the precipitate was evaporated to a small bulk on a water-bath, alcohol added, and the precipitate thoroughly washed with alcohol, first by decantation and then on a filter. It was then finally ignited until of constant weight.

(1) 0·7083 gramme of the salt lost 0·0340 gramme at 110° C., corresponding to 4·80 per cent. of water, and gave 0·2818 gramme $Mg_2 P_2 O_7$, corresponding to 0·2481 gramme, or 35·03 per cent. $H_3 P O_4$.

(2) 0·7370 gramme of the salt lost 0·0360 gramme at 110° C., corresponding to 4·88 per cent. of water, and gave 0·2988 gramme $Mg_2 P_2 O_7$, corresponding to 0·2631 gramme, or 35·70 per cent. $H_3 P O_4$.

(3) 0·2268 gramme of the salt gave 0·0392 gramme of platinum corresponding to 0·1348 gramme, or 59·43 per cent. of berberine.

(4) 0·1868 gramme of the salt gave 0·0326 gramme of platinum, corresponding to 0·1121 gramme, or 60·01 per cent. of berberine.

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[1] Parsons and Wrampelmeier note that the salt obtained by them by the use of acid calcium phosphate readily absorbed water, but not so readily when re-crystallised.

These results may be tabulated as follows :—

		(1)	(2)	(3)	(4)	Calculated for $C_{20}H_{17}NO_4 \cdot 2H_3PO_4 + 1\frac{1}{2}H_2O$
H_2O	=	4·80	4·88	—	—	4·83 per cent.
H_3PO_4	=	35·03	35·70	—	—	35·13 ,,
$C_{20}H_{17}NO_4$	=	—	—	59·43	60·01	60·04 ,,
						100·00

Some experiments were made to ascertain whether the base could be accurately estimated by means of the crystalline chloroform compound, $C_{20}H_{17}NO_4 \cdot CHCl_3$. For this purpose a weighed portion of berberine phosphate was dissolved in water, an excess of soda added, and the base extracted by shaking out with chloroform. The chloroform solution was evaporated, and the residue, when dry, heated in a water oven till of constant weight. Similar experiments were made with berberine nitrate and sulphate, but in no case were concordant results obtainable by this method, the difference being as much as 1 per cent.

It was now thought of interest to prepare some berberine phosphate according to the method described by Parsons and Wrampelmeier (loc. cit.). After the removal of the product, and allowing the mixed mother liquor and alcoholic washings to stand over night, a precipitate separated out. On filtering this off it was found to be quite white, and to consist of calcium phosphate with traces of sulphate.

The berberine phosphate thus obtained was of a bright yellow colour, and did not absorb water when exposed to the air, but darkened slightly at 110° C. It contained some calcium, and on recrystallising from alcohol left about 5 per cent. of a white residue consisting of calcium phosphate and sulphate. The recrystallised salt was in the form of bright yellow, silky needles, and was free from calcium and sulphates. Analyses were made of the salt both before and after recrystallisation.

The salt containing calcium was treated with sulphuric acid and potassium nitrate as before described, the liquid diluted, made alkaline with ammonia, and acidified with acetic acid. The calcium was then estimated by precipitation with ammonium oxalate in the usual way.

0·8090 gramme of the salt lost 0·0272 gramme at 110° C., corresponding to 3·36 per cent. of water. The same quantity gave 0·0294 gramme $CaCO_3 = 0·0117$ gramme Ca, or 1·45 per cent. Ca.,

and also gave 0·4011 gramme $Mg_2 P_2 O_7$ = 0·3532 gramme $H_3 P O_4$, or 43·66 per cent. $H_3 P O_4$.

0·8618 gramme of the salt lost 0·0290 gramme at 110° C., corresponding to 3·36 per cent. of water. The same quantity gave 0·0374 gramme $Ca C O_3$ = 0·0149 gramme Ca, or 1·74 per cent. Ca, and also gave 0·4301 gramme $Mg_2 P_2 O_7$ = 0·3787 gramme $H_3 P O_4$, or 43·94 per cent. $H_3 P O_4$.

1·0796 gramme was treated with sulphuric acid and potassium nitrate as before described, the liquid diluted, made alkaline with ammonia, then acidified with acetic acid, and made up to 250° c.c. when cold. Portions of this were titrated with standard uranium nitrate solution, of which 1 c.c. = 0·005 gramme $P_2 O_5$, or 0·0069 gramme $H_3 P O_4$. One portion of 50 c.c. required 13·91 c.c. of uranium solution, another 50 c.c. required 14·05 c.c. of uranium solution; the mean of the two is 13·98 c.c. = 0·0965 gramme $H_3 P O_4$, or 44·68 per cent. $H_3 P O_4$.

On account of the contamination of this salt with calcium, it is obviously useless to attempt to calculate a chemical formula from the above results; but they nevertheless serve to show that the amount of phosphoric acid contained in this product is also not in accordance with the formula suggested by Parsons and Wrampelmeier, for a salt obtained by the method employed by them— viz., $C_{20} H_{17} N O_4 \cdot 7 H_3 P O_4 \cdot 4 H_2 O$, which would require 6·59 per cent. of water and 62·76 per cent. $H_3 P O_4$.

The same salt, but purified by recrystallisation, and free from calcium, as previously noted, was now analysed. The water of crystallisation was determined by heating to 110° C., until of constant weight, the phosphoric acid gravimetrically, as with the first salt described, and the base by precipitation with platinic chloride.

(1) 0·3834 gramme of the salt lost 0·0103 gramme at 110° C. corresponding to 2·68 per cent. of water and gave 0·1589 gramme $Mg_2 P_2 O_7$, corresponding to 0·1399 gramme or 36·49 per cent. $H_3 P O_4$.

(2) 0·5053 gramme of the salt lost 0·0136 gramme at 110° C., corresponding to 2·69 per cent. of water, and gave 0·2083 gramme $Mg_2 P_2 O_7$, corresponding to 0·1834 gramme or 36·30 per cent. $H_3 P O_4$.

(3) 0·2052 gramme of the salt gave 0·0362 gramme of platinum, corresponding to 0·1244 gramme or 60·62 per cent. of berberine.

(4) 0·1920 gramme of the salt gave 0·0338 gramme of plati-

num, corresponding to $0\cdot1162$ gramme or $60\cdot5\underline{2}$ per cent. of berberine.

		(1)	(2)	(3)	(4)	Calculated for $C_{20} H_{17} N O_4 \cdot 2 H_3 P O_4 + H_2 O$
$H_2 O$	$=$	$2\cdot68$	$2\cdot69$	—	—	$3\cdot28$ per cent.
$H_3 P O_4$	$=$	$36\cdot49$	$36\cdot30$	—	—	35.70 ,,
$C_{20} H_{17} N O_4 =$		—	—	$60\cdot62$	$60\cdot52$	$61\cdot02$,,
						$100\cdot00$

From these results it will be seen that the berberine phosphate prepared by me, either by treating pure berberine-acetone with an excess of phosphoric acid, or by the interaction of mono-berberine sulphate and acid calcium phosphate, and subsequent purification of the product, has the composition $C_{20} H_{17} N O_4 \cdot 2 H_3 P O_4$, with varying amounts of water of crystallisation.

It thus agrees with the results obtained by Parsons in his analysis of a pure salt prepared by Willmarth, but, as was anticipated, disproves the formula $C_{20} H_{17} N O_4 \cdot 7 H_3 P O_4 + 4 H_2 O$ assigned by Parsons and Wrampelmeier to a salt prepared by the interaction of berberine sulphate and acid calcium phosphate, notwithstanding the fact that one of my salts was prepared by the method followed by them, although carefully purified from calcium before analysis, and that we have employed the same methods for the determination of the base and the acid.

The latter investigators had themselves remarked that " this formula seems, at first sight, an improbable one," and the support that was given to it by the subsequent experiments of Coblentz cannot be considered altogether satisfactory or conclusive, for although he worked with the pure alkaloid and an excess of phosphoric acid, he appears to have made no direct determination of the base, and only determined the amount of phosphoric acid present by indirect methods.

It will be seen that my results also do not confirm the formula given by Schmidt, viz. : $(C_{20} H_{17} N O_4)_3 (H_3 P O_4)_2 + 5 H_2 O$, but, as previously noted, not having been able to find any record of analytical data relating to this formula, it can only incidentally be referred to.

The accuracy of the determinations of the base by means of platinic chloride I have confirmed by control experiments with pure berberine salts of well-established composition, such, for example, as the nitrate and sulphate, which were likewise prepared by myself.

It was thought of interest to compare the results obtained by this method with the less accurate determinations of the berberine by weighing it in the form of the berberine-chloroform compound, and these are presented in the following table :—

Berberine Salt used. B = Berberine, $C_{20} H_{17} N O_4$.	Weight of Salt taken.	Weight of Chloroform Compound obtained.	Weight of Platinum Residue.	Weight of corresponding amount of base.	Percentage of base found.	Percentage of base calculated $C_{20} H_{17} N O_4 = 335$, $Pt = 194 \cdot 8$.
$B \cdot H N O_3$	0·5274	0·6074	—	0·4474	84·90	}84·16
	0·1956	—	0·0479	0·1647	84·20	
$B \cdot H_2 S O_4$	0·3831	0·4118	—	0·3035	79·23	
	0·3384	0·3594	—	0·2649	78·29	}77·36
	0·1953	—	0·0441	0·1516	77·62	
$B \cdot 2 H_3 P O_4 + 1\frac{1}{2} H_2 O$.	0·6574	0·5312	—	0·3915	59·56	
(From acetone compound	0·5826	0·4788	—	0·3529	60·56	}60·04
with phosphoric acid) .	0·2268	—	0·0392	0·1348	59·43	
	0·1868	—	0·0326	0·1121	60·01	
$B \cdot 2 H_3 P O_4 + 1 H_2 O$.	0·2052	—	0·0362	0·1244	60·62	
From $B \cdot H_2 S O_4$ and $Ca H_4$ $(P O_4)_2$ (re-crystallised) .	0·1920	—	0·0338	0·1162	60·52	61·01

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